FORM PTO-1390 (Modified) (REV 10-95) U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE ATTORNEY'S DOCKET NUMBER

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER: 00960-514

U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR): 09/445328

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/US98/03197	5 May 1998	5 May 1997

TITLE OF INVENTION

THERAPIES FOR ACUTE RENAL FAILURE

APPLICANT(S) FOR DO/EO/US

SAMPATH, Kuber T.; COHEN, Charles M.

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This is an express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. ☒ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. ☒ A copy of the International Application as filed (35 U.S.C. 371 (c) (2))
   a. ☐ is transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ has been transmitted by the International Bureau.
   c. ☒ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☐ A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. ☒ A copy of the International Search Report (PCT/ISA/210).
8. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371 (c)(3))
   a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ have been transmitted by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☒ have not been made and will not be made.
9. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
10. ☒ An oath or declaration of the inventor(s) (35 U.S.C. 371 (c)(4)).
11. ☒ A copy of the International Preliminary Examination Report (PCT/IPEA/409).
12. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371 (c)(5)).

Items 13 to 18 below concern document(s) or information included:

13. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
14. ☒ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
15. ☐ A **FIRST** preliminary amendment.
    A **SECOND** or **SUBSEQUENT** preliminary amendment.
16. ☐ A substitute specification.
17. ☐ A change of power of attorney and/or address letter.
18. ☒ Certificate of Mailing by Express Mail
19. ☒ Other items or information:

Small Entity Statement; Petition to Revive Unintentional Abandonment and Fee

Express Mailing Label No.: EM515800173US
Deposited: 7 December 1999

U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR)	INTERNATIONAL APPLICATION NO.	ATTORNEY'S DOCKET NUMBER
09/445328	PCT/US98/03197	00960-514

20. The following fees are submitted:.

CALCULATIONS PTO USE ONLY

BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)) :

- ☒ Search Report has been prepared by the EPO or JPO **$930.00**
- ☐ International preliminary examination fee paid to USPTO (37 CFR 1.482) **$720.00**
- ☐ No international preliminary examination fee paid to USPTO (37 CFR 1.482) but international search fee paid to USPTO (37 CFR 1.445(a)(2)) **$790.00**
- ☐ Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2) paid to USPTO **$1,070.00**
- ☐ International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(2)-(4) **$98.00**

ENTER APPROPRIATE BASIC FEE AMOUNT = **$930.00**

Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☒ 30 months from the earliest claimed priority date (37 CFR 1.492 (e)). **$130.00**

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE	
Total claims	115 - 20 =	95	x $22.00	**$2,090.00**
Independent claims	8 - 3 =	5	x $82.00	**$410.00**
Multiple Dependent Claims **(check if applicable)**.			☒	**$270.00**

TOTAL OF ABOVE CALCULATIONS = **$3,830.00**

Reduction of 1/2 for filing by small entity, if applicable. Verified Small Entity Statement must also be filed (Note 37 CFR 1.9, 1.27, 1.28) **(check if applicable)**. ☒ **$1,915.00**

SUBTOTAL = **$1,915.00**

Processing fee of **$130.00** for furnishing the English translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492 (f)). + **$0.00**

TOTAL NATIONAL FEE = **$1,915.00**

Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31) **(check if applicable).** ☐ **$0.00**

TOTAL FEES ENCLOSED = **$1,915.00**

Amount to be: refunded	$
charged	$

☒ A check in the amount of **$1,915.00** to cover the above fees is enclosed.

☐ Please charge my Deposit Account No. in the amount of to cover the above fees.
A duplicate copy of this sheet is enclosed.

☒ The Commissioner is hereby authorized to charge any fees which may be required, or credit any overpayment to Deposit Account No. **50-0311** A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

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Boston, Massachusetts 02111
United States of America

SIGNATURE

Ivor R. Elrifi
NAME

39,529
REGISTRATION NUMBER

7 December 1999
DATE

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANTS:	Cohen and Sampath		
ASSIGNEE:	CREATIVE BIOMOLECULES		
SERIAL NUMBER:	Claims priority to PCT/US98/03197	EXAMINER:	Not Yet Assigned
FILING DATE:		ART UNIT:	Not Yet Assigned
FOR:	THERAPIES FOR ACUTE RENAL FAILURE		

STATEMENT CLAIMING SMALL ENTITY STATUS
(37 CFR 1.9(f) and 1.27(c) – SMALL BUSINESS CONCERN)

I hereby state that I am an official of the small business concern empowered to act on behalf of the concern identified below:

CREATIVE BIOMOLECULES, INC.
101 Huntington Ave., Suite 2400
Boston, MA 02199

I hereby state that the above identified small business concern qualifies as a small business concern, as defined in 13 C.F.R. § 121, and reproduced in 37 CFR 1.9(d), for purposes of paying reduced fees to the United States Patent and Trademark Office under Sections 41(a) and (b) of Title 35, United States Code, in that the number of employees of the concern, including those of its affiliates, does not exceed 500 persons. For purposes of this statement, (1) the number of employees of the business concern is the average over the previous fiscal year of the concern of the persons employed on a full-time, part-time or temporary basis during each of the pay periods of the fiscal year, and (2) concerns are affiliates of each other when either, directly or indirectly, one concern controls or has the power to control the other, or a third-party or parties controls or has the power to control both.

I hereby state that rights under contract or law have been conveyed to, and remain with, the small business concern identified above with regard to the invention described in:

- ☒ the specification filed herewith with title as listed above;
- ☐ the application identified above; or
- ☐ the patent identified above.

If the rights held by the above identified small business concern are not exclusive, each individual, concern or organization having rights in the invention is listed below and no rights to the invention are held by any person, other than the inventor, who would not qualify as an independent inventor under 37 C.F.R. § 1.9(c), if that person made the invention, or by any concern which would not qualify as a small business concern under 37 C.F.R. § 1.9(d) or a nonprofit organization under 37 C.F.R. § 1.9(e).

APPLICANTS: Cohen and Sampath

Each person, concern, or organization having any rights in the invention is listed below:

☒ no such person, concern, or organization exists.

☐ each such person, concern, or organization is listed below: *

* ***Note:*** *Separate Statements Claiming Small Entity Status are required from each named person, concern, or organization having rights to the invention. 37 C.F.R. § 1.27.*

Full Name:
Address:
☐ Individual ☐ Small Business Concern ☐ Non-profit Organization

I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small business entity is no longer appropriate. 37 C.F.R. § 1.28(b).

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

Name of person signing: **Cheryl Lawton**

Title in organization of person signing: **Vice President, General Counsel**

Address of person signing: CREATIVE BIOMOLECULES, INC.
101 Huntington Ave., Suite 2400
Boston, MA 02199

Signature: _____ Date: 12/1/99

TRADOCS:1262410.1(R22Y01!.DOC)

THERAPIES FOR ACUTE RENAL FAILURE

Field of the Invention

The present invention relates generally to methods of treatment for renal disease and, in particular, methods of treatment for mammals, including humans, afflicted with, or at risk of, acute renal failure. The methods involve the administration of certain proteins of the osteogenic protein/bone morphogenetic protein (OP/BMP) family within the TGF-β superfamily of proteins.

Background of the Invention

The mammalian renal system serves primary roles both in the removal of catabolic waste products from the bloodstream and in the maintenance of fluid and electrolyte balances in the body. Renal failure is, therefore, a life-threatening condition in which the build-up of catabolites and other toxins, and/or the development of significant imbalances in electrolytes or fluids, may lead to the failure of other major organs systems and death. As a general matter, renal failure is classified as "acute" or "chronic" As detailed below, acute renal failure typically involves a rapid, drastic, life-threatening loss of renal function over a period of a few hours to several weeks. In contrast, chronic renal failure typically involves a slow, progressive loss of renal function over a period of months to years, during which time the subject's life is not immediately threatened.

Acute renal failure is characterized by an abrupt cessation or substantial reduction of renal function, and is typically diagnosed by relatively rapid increases in blood urea nitrogen (BUN) or serum creatinine levels over a period of a few hours or days. In as many as 90-95% of cases, acute renal failure may be secondary to trauma, surgery or another acute medical condition. Generally speaking, acute renal failure may be due to pre-renal , post-renal, or intrinsic renal causes. Pre-renal causes (e.g., decreased cardiac output, hypovolemia, altered vascular resistance) and post-renal causes (e.g., obstructions or constrictions of the ureters, bladder or urethra) do not involve direct damage to the kidneys but, by affecting the flow of blood to the kidneys or the flow of urine from the kidneys, may lead to significant permanent and/or progressive damage to renal tissues. On the other hand, acute renal failure may be due to intrinsic renal causes which involve a more direct insult or injury to the kidneys, and which also may entail permanent and/or progressive damage to the nephrons or other kidney structures. Intrinsic causes of acute renal failure include but are not limited to infectious diseases (e.g., various bacterial, viral or parasitic infections), inflammatory diseases (e.g., glomerulonephritis, systemic lupus erythematosus), ischemia (e.g., renal artery occlusion), toxic syndromes (e.g., heavy metal poisoning, side-effects of antimicrobial treatments or chemotherapy), and direct traumas.

In human acute renal failure patients, oliguria (urine output < 400 ml/day) or anuria (urine output < 50 ml/day) may be present in 50-70% of cases, BUN levels may climb 10-20 mg/dL/day or faster, plasma creatinine levels may climb 0.5-1.0 mg/dL/day, and metabolic acidosis is almost always present. If not treated, the electrolyte and fluid imbalances (e.g., hyperkalemia, acidosis, edema) associated with acute renal failure may lead to life-threatening arrhythmia, congestive heart failure, or multiple organ system failures. Due to the severity of acute renal failure, episodes rarely last longer than several weeks without mortality and are treated on an in-patient basis.

A need remains, therefore, for treatments which will prevent, inhibit, delay or alleviate the permanent or progressive loss of renal function which may result from acute renal failure.

Summary of the Invention

The present invention is directed to methods of treatment, and pharmaceutical preparations for use in the treatment, of mammalian subjects in, or at risk of, acute renal failure. Such subjects include subjects already in acute renal failure, as well as any subject reasonably expected to suffer acute loss of renal function. Whether a particular subject is in acute renal failure, or is at risk of acute renal failure, is a determination which may routinely be made by one of ordinary skill in the relevant medical or veterinary art. Subjects in acute renal failure include those showing either (1) an increase in blood urea nitrogen (BUN) at a rate of at least 2 to 4 mmol/L/day (5 to 10 mg/dL/day), or (2) an increase in serum creatinine at a rate of at least 20 to 40 μmol/L/day (0.25 to 0.5 mg/dL/day). More typically, subjects in acute renal failure show rates of increase in BUN of at least 4 to 8 mmol/L/day (10 to 20 mg/dL/day), and rates of increase of serum creatinine of at least 40 to 80 μmol/L/day (0.5 to 1.0 mg/dL/day). Subjects "at risk" of acute renal failure include subjects which are reasonably expected to enter acute renal failure or which are otherwise expected to otherwise suffer a rapid progressive loss of renal function. Whether a particular subject is at risk is a determination which may routinely be made by one of ordinary skill in the relevant medical or veterinary art. Subjects at risk of acute renal failure include but are not limited to the following: (1) subjects in which serial determination of BUN indicates a rate of increase of at least 1 to 2 mmol/L/day (2.5 to 5 mg/dL/day); (2) subjects in which serial determination of serum creatinine indicates a rate of increase of at least 10 to 20 μmol/L/day (0.125 to 0.25 mg/dL/day); (3) subjects which have been diagnosed with a pre-renal cause of acute renal failure; (4) subjects which have been diagnosed with a post-renal cause of acute renal failure; and (5) subjects which have been diagnosed with an intrinsic renal cause of acute renal failure.

The methods and compositions of this invention capitalize in part upon the discovery that certain proteins of eukaryotic origin, defined herein as OP/BMP renal therapeutic agents, and including members of the osteogenic protein/bone morphogenetic protein (OP/BMP) family of proteins, may be used in the treatment of subjects in, or at risk of, acute renal failure. Useful renal therapeutic agents include polypeptides, or functional variants of polypeptides, comprising at least

the C-terminal six- or seven-cysteine domain of a mammalian protein selected from the group consisting of OP-1, OP-2, OP-3, BMP2, BMP3, BMP4, BMP5, BMP6, BMP9, and proteins which exhibit at least 70% or, more preferably, 75% or 80% amino acid sequence homology with the amino acid sequence of the seven-cysteine domain of human OP-1; and which are (a) capable of inducing chondrogenesis in the Reddi-Sampath ectopic bone assay (Sampath and Reddi (1981), Proc. Natl. Acad. Sci. (USA) 78:7599-7603) or a substantially equivalent assay, (b) capable of significantly preventing, inhibiting, delaying or alleviating the permanent or progressive loss of renal function which may result from acute renal failure in a standard animal model of acute renal failure, or (c) capable of causing a clinically significant improvement in a standard marker of renal function when administered to a mammal in, or at risk of, acute renal failure.

The renal therapeutic agents of the invention may be administered by any route of administration which is compatible with the selected agent, and may be formulated with any pharmaceutically acceptable carrier appropriate to the route of administration. Preferred routes of administration are parenteral and, in particular, intravenous, intraperitoneal, and renal intracapsular. Administration is expected to be continuous or frequent (e.g., daily) during the period of acute renal failure, typically 1-3 weeks, but may also be continued for several weeks or months after the acute phase. Daily dosages of the renal therapeutic agents are expected to be in the range of about 0.01-1000 μg/kg body weight, and more preferably about 10-700 μg/kg body weight, although precise dosages will vary depending upon the particular renal therapeutic agent employed and the particular subject's medical condition and history.

The treatments of the present invention are useful in reducing the rate and/or degree of morbidity in mammals in, or at risk of, acute renal failure. In addition, the treatments of the present invention are useful in preventing, inhibiting, delaying or alleviating the permanent or progressive loss of renal function which may result from acute renal failure. As such, the present invention has great value, not only for increasing survival rates, but also in preventing or delaying the need for chronic dialysis or renal replacement therapy, in preventing or delaying the development of chronic renal insufficiency or chronic renal failure, and/or for reducing the necessary frequency of chronic renal dialysis.

In another aspect, the OP/BMP renal therapeutic agents of the invention are useful in reducing inflammation and neutrophil-mediated tissue damage. In this respect, the OP/BMP therapeutic agents are also shown to be useful in reducing the expression of ICAMs, particularly ICAM-1, when administered systemically. In particular, the OP/BMP therapeutics may be used to reduce ICAM expression in epithelial issue, particularly renal epithelium.

In yet another aspect, the OP/BMP renal therapeutic agents of the invention are useful for inhibiting apoptosis of cells in damaged or injured tissues. In this respect, the OP/BMP therapeutic agents are shown to reduce apoptosis of damaged or injured epithelial tissues, particularly renal epithelium.

In another aspect, the OP/BMP renal therapeutic agents of the invention may be used in the manufacture of medicaments for the treatment of any of the above-described conditions.

Brief Description of the Drawings

FIGURE 1 is a bar graph showing the effect of OP-1 on creatinine levels in rats in which ischemic renal damage was induced by temporarily preventing renal blood flow. The vertical axis of the graph is a scale of creatinine (μmol/L).

FIGURE 2 is a table showing the effect of OP-1 on creatinine levels and on hemodynamic parameters in rats in which renal function was impaired by administering norepinephrine.

Detailed Description of the Invention

I. Definitions

In order to more clearly and concisely point out the subject matter of the claimed invention, the following definitions are provided for specific terms used in the following written description and appended claims.

Acute Renal Failure. Acute renal failure is typically defined as a rapid deterioration in renal function sufficient to result in the accumulation of nitrogenous wastes in the body (see, e.g., Anderson and Schrier (1994), in Harrison's Principles of Internal Medicine, 13th edition, Isselbacher et al., eds., McGraw Hill Text, New York). Rates of increase in BUN of at least 4 to 8 mmol/L/day (10 to 20 mg/dL/day), and rates of increase of serum creatinine of at least 40 to 80 μmol/L/day (0.5 to 1.0 mg/dL/day), are typical in acute renal failure. In subjects which are catabolic (or hypercatabolic), rates of increase in BUN may exceed 100/mg/dL/day. Rates of increase in BUN or serum creatinine are usually determined by serial blood tests and, preferably, at least two blood tests are conducted over a period of between 6 and 72 hours or, more preferably, 12 and 24 hours. A distinction is sometime made between "acute" renal failure (deterioration over a period of days) and "rapidly progressive" renal failure (deterioration over a period of weeks). As used herein, however, the phrase "acute renal failure" is intended to embrace both syndromes.

Pre-renal Causes of Acute Renal Failure. As used herein, pre-renal causes of acute renal failure include decreased cardiac output, hypovolemia, volume redistribution, and altered vascular resistance.

Post-renal Causes of Acute Renal Failure. As used herein, post-renal causes of acute renal failure include ureteral, pelvic and bladder obstructions. For example, blood clots and kidney stones may cause obstructions of the ureters or bladder. Obstructions also may arise from sloughed papillae, and fungus balls. Extrinsic obstructions may result from, for example,

malignancies or hypertrophies (e.g., prostatic or bladder carcinoma), retroperitoneal fibrosis, or iatrogenic causes (e.g., inadvertent ligation). Urethral strictures or phimosis may also cause pre-renal acute renal failure.

Intrinsic Renal Causes of Acute Renal Failure. As used herein, intrinsic renal causes of acute renal failure include:

(1) Abnormalities of the vasculature such as vasoconstrictive disease (e.g., malignant hypertension, scleroderma, hemolytic uremic syndrome, thrombotic thrombocytopenic purpura) and vasculitis (e.g., polyarteritis nodosa, hypersensitivity angiitis, serum sickness, Wegener's granulomatosis, giant cell arteritis, mixed cryoglobulinemia, Henoch-Schönlein purpura, systemic lupus erythematosus);

(2) Abnormalities of the glomeruli such as post-infectious abnormalities (e.g., post-streptococcal, pneumococcal, gonococcal, staphylococcal, enterococcal, viral [e.g., hepatitis B and C, mumps, measles, Epstein-Barr], malarial, or related to brucellosis, Legionella, Listeria, shunt nephritis, leprosy, leptospirosis, or visceral abscesses) and non-infectious abnormalities (e.g., rapidly progressive glomerulonephritis, membranoproliferative glomerulonephritis, Goodpasture's syndrome, systemic lupus erythematosus, Wegener's granulomatosis);

(3) Acute interstitial nephritis resulting from drug related causes (e.g., penicillins, sulfonamides, carbenicillin, cephalosporin, erythromycin, nafcillin, oxacillin, nonsteroidal anti-inflammatory agents, diuretics [furosemide, ethacrynic acid, thiazide, spironolactone, mercurials], phenytoin, phenobarbital, probenicid, allopurinol, cimetidine), infection related causes (e.g., acute pyelonephritis, streptococcal, staphylococcal, leptospirosis, malaria, salmonellosis), papillary necrosis (e.g., associated with diabetes mellitus, sickle cell diseases, analgesic abuse, alcoholism), and other, miscellaneous causes (e.g., sarcoidosis, leukemia, lymphoma);

(4) Intratubular obstruction from crystal deposition (e.g., uric acid, oxalate, methotrexate) or multiple myeloma and light chain disease; and

(5) Acute tubular necrosis resulting from nephrotoxins (e.g., antimicrobials such as aminoglycosides, tetracyclines, amphotericin, polymyxin, cephalosporins), heavy metals (e.g., mercury, lead, arsenic, gold salts, barium), and other, miscellaneous chemical agents (e.g., cisplatin, doxorubicin, streptozocin, methoxyflurane, halothane, ethylene glycol, carbon tetrachloride), or from ischemia (e.g., hemorrhage, hypotension, sepsis, burns, renal infarction, renal artery dissection, rhabdomyolysis, trauma), or other miscellaneous causes (e.g., contrast agents, transfusion reactions, myoglobinemia, heat stroke, snake and spider bites).

Other diseases and conditions which place a subject at risk of acute renal failure include: kidney transplantation surgery (as donor or recipient), bilateral arterial occlusion, bilateral acute renal vein thrombosis, acute uric acid nephropathy, hypovolemia, cardiovascular collapse, acute bilateral upper tract obstruction, hypercalcemic nephropathy, hemolytic uremic syndrome, acute urinary retention, malignant nephrosclerosis, essential mixed cyroimmunoglobulinemia, oxalate

nephropathy, cortical necrosis, postpartum glomerulosclerosis, hypersensitivity nephropathy, scleroderma, idiopathic rapidly progressive glomerulonephritis, Goodpasture's syndrome, non-Goodpasture's anti-GBM disease, acute bacterial endocarditis or visceral sepsis, microscopic polyarteritis nodosa, Wegener's granulomatosis, allergic granulomatosis, acute radiation nephritis, post-streptococcal glomerulonephritis, nonstreptococcal post-infectious glomerulonephritis, diffuse proliferative lupus nephritis, membranoproliferative glomerulonephritis, renal vein thrombosis, Waldenstrom's macroglobulinemia, multiple myeloma, Berger's (IgA) nephropathy, Henoch-Schönlein purpura, and focal glomerulosclerosis.

OP/BMP renal therapeutic agent. As used herein, the terms "OP/BMP renal therapeutic agent," "renal therapeutic agent of the invention," and the like mean a polypeptide, or a functional variant of a polypeptide, comprising at least the C-terminal six- or seven-cysteine domain of a mammalian protein selected from the group consisting of OP-1, OP-2, OP-3, BMP2, BMP3, BMP4, BMP5, BMP6, BMP9, and proteins which exhibit at least 70% or, more preferably, 75% or 80% amino acid sequence homology with the amino acid sequence of the seven-cysteine domain of human OP-1; and which is (a) capable of inducing chondrogenesis in the Reddi-Sampath ectopic bone assay (Sampath and Reddi (1981), Proc. Natl. Acad. Sci. (USA) 78:7599-7603) or a substantially equivalent assay, (b) capable of significantly preventing, inhibiting, delaying or alleviating the permanent or progressive loss of renal function which may result from acute renal failure in a standard animal model of acute renal failure, or (c) capable of causing a clinically significant improvement in a standard marker of renal function when administered to a mammal in, or at risk of, acute renal failure.

Therapeutic efficacy. As used herein, a renal therapeutic agent of the invention is said to have "therapeutic efficacy," and an amount of the agent is said to be "therapeutically effective," if administration of that amount of the agent is sufficient to cause a clinically significant improvement in a standard marker of renal function when administered to a mammalian subject (e.g., a human patient) in, or at risk of, acute renal failure. Such markers of renal function are well known in the medical literature and include, without being limited to, rates of increase in BUN levels, rates of increase in serum creatinine, static measurements of BUN, static measurements of serum creatinine, glomerular filtration rates (GFR), ratios of BUN/creatinine, serum concentrations of sodium (Na^+), urine/plasma ratios for creatinine, urine/plasma ratios for urea, urine osmolality, daily urine output, and the like (see, for example, Anderson and Schrier (1994), in Harrison's Principles of Internal Medicine, 13th edition, Isselbacher et al., eds., McGraw Hill Text, New York; Kumar and Stein (1994), in Internal Medicine, 4th Edition, J.H. Stein, ed., Mosby-Year Book, Inc. St. Louis.)

II. Embodiments of the Invention

A. General

The present invention depends, in part, upon the surprising discovery that administration of certain protein-based renal therapeutic agents to subjects in acute renal failure, or at risk of acute renal failure, can reduce mortality and/or morbidity rates, and prevent, inhibit, delay or alleviate the permanent and/or progressive loss of renal function associated with acute renal failure. The present invention is particularly surprising in light of the fact that the agents of the present invention are proteins, whereas standard treatment regimes for acute renal failure include the limitation of protein intake to reduce strain on the kidneys. In preferred embodiments, the renal therapeutic agents of the invention are members of the osteogenic protein/bone morphogenetic protein (OP/BMP) family within the TGF-β superfamily of proteins.

B. OP/BMP Renal Therapeutic Agents

The renal therapeutic agents of the present invention are naturally occurring proteins, or functional variants of naturally occurring proteins, in the osteogenic protein/bone morphogenetic protein (OP/BMP) family within the TGF-β superfamily of proteins. That is, these proteins form a distinct subgroup, referred to herein as the "OP/BMP family," within the loose evolutionary grouping of sequence-related proteins known as the TGF-β superfamily. Members of this protein family comprise secreted polypeptides that share common structural features, and that are similarly processed from a pro-protein to yield a carboxy-terminal mature protein. Within the mature protein, all members share a conserved pattern of six or seven cysteine residues defining a 97-106 amino acid domain, and the active form of these proteins is either a disulfide-bonded homodimer of a single family member, or a heterodimer of two different members (see, e.g., Massague (1990), Annu. Rev. Cell Biol. 6:597; Sampath et al. (1990), J. Biol. Chem. 265:13198). For example, in its mature, native form, natural-sourced human OP-1 is a glycosylated dimer typically having an apparent molecular weight of about 30-36 kDa as determined by SDS-PAGE. When reduced, the 30 kDa protein gives rise to two glycosylated peptide subunits having apparent molecular weights of about 16 kDa and 18 kDa. The unglycosylated protein has an apparent molecular weight of about 27 kDa. When reduced, the 27 kDa protein gives rise to two unglycosylated polypeptide chains, having molecular weights of about 14 kDa to 16 kDa.

Typically, the naturally occurring OP/BMP proteins are translated as a precursor, having an N-terminal signal peptide sequence, a "pro" domain, and a "mature" protein domain. The signal peptide is typically less than 30 residues, and is cleaved rapidly upon translation at a cleavage site that can be predicted using the method of Von Heijne (1986), Nucleic Acids Research 14:4683-4691. The "pro" domain is variable both in sequence and in length, ranging from approximately 200 to over 400 residues. The pro domain is cleaved to yield the "mature" C-terminal domain of approximately 115-180 residues, which includes the conserved six- or

seven-cysteine C-terminal domain of 97-106 residues. As used herein, the "pro form" of an OP/BMP family member refers to a protein comprising a folded pair of polypeptides, each comprising a pro domain in either covalent or noncovalent association with the mature domains of the OP/BMP polypeptide. Typically, the pro form of the protein is more soluble than the mature form under physiological conditions. The pro form appears to be the primary form secreted from cultured mammalian cells. The "mature form" of the protein refers to mature C-terminal domain which is not associated, either covalently or noncovalently, with the pro domain. Any preparation of OP-1 is considered to contain mature form when the amount of pro domain in the preparation is no more than 5% of the amount of "mature" C-terminal domain.

OP/BMP family members useful herein include any of the known naturally-occurring native proteins including allelic, phylogenetic counterpart and other variants thereof, whether naturally-sourced or biosynthetically produced (e.g., including "muteins" or "mutant proteins"), as well as new, active members of the OP/BMP family of proteins.

Particularly useful sequences include those comprising the C-terminal seven cysteine domains of mammalian, preferably human, OP-1, OP-2, OP-3, BMP2, BMP3, BMP4, BMP5, BMP6, BMP8 and BMP9. Other proteins useful in the practice of the invention include active forms of GDF-5, GDF-6, GDF-7, DPP, Vg1, Vgr-1, 60A, GDF-1, GDF-3, GDF-5, GDF-6, GDF-7, BMP10, BMP11, BMP13, BMP15, UNIVIN, NODAL, SCREW, ADMP or NURAL and amino acid sequence variants thereof. In one currently preferred embodiment, the renal therapeutic agents of the invention are selected from any one of: OP-1, OP-2, OP-3, BMP2, BMP3, BMP4, BMP5, BMP6, and BMP9.

Publications disclosing these sequences, as well as their chemical and physical properties, include: OP-1 and OP-2: U.S. Pat. No. 5,011,691, U.S. Pat. No. 5,266,683, and Ozkaynak et al. (1990), EMBO J. 9:2085-2093; OP-3: WO94/10203; BMP2, BMP3, and BMP4: U.S. Pat. No. 5,013,649, W091/18098, WO88/00205, and Wozney et al. (1988), Science 242:1528-1534; BMP5 and BMP6: WO90/11366 and Celeste et al. (1991), Proc. Natl. Acad. Sci. (USA) 87:9843-9847; Vgr-1: Lyons et al. (1989), Proc. Natl. Acad. Sci. (USA) 86: 4554-4558; DPP: Padgett et al. (1987), Nature 325:81-84; Vgl: Weeks (1987), Cell 51:861-867; BMP-9: WO95/33830; BMP10: WO94/26893; BMP-11: WO94/26892; BMP12: WO95/16035; BMP-13: WO95/16035; GDF-1: WO92/00382 and Lee et al. (1991), Proc. Natl. Acad. Sci. (USA) 88:4250-4254; GDF-8: WO94/21681; GDF-9: WO94/15966; GDF-10: WO95/10539; GDF-11: WO96/01845; BMP-15: WO96/36710; MP121: WO96/01316; GDF-5 (CDMP-1, MP52): WO94/15949, WO96/14335, WO93/16099 and Storm et al. (1994), Nature 368:639-643; GDF-6 (CDMP-2, BMP13): WO95/01801, WO96/14335 and WO95/10635; GDF-7 (CDMP-3, BMP12): WO95/10802 and WO95/10635; BMP-3b: Takao, et al. (1996), Biochem. Biophys. Res. Comm. 219:656-662; GDF-3: WO94/15965; 60A: Blaster et al. (1993), Cell 73:687-702 and GenBank accession number L12032. In another embodiment, useful proteins

include biologically active biosynthetic constructs, including novel biosynthetic proteins and chimeric proteins designed using sequences from two or more known OP/BMP family proteins. See also the biosynthetic constructs disclosed in U.S. Pat. No. 5,011,691, the disclosure of which is incorporated herein by reference (e.g., COP-1, COP-3, COP-4, COP-5, COP-7, and COP-16).

In other preferred embodiments, the renal therapeutic agents useful herein include therapeutically effective proteins in which the amino acid sequences comprise a sequence sharing at least 70% amino acid sequence "homology" and, preferably, 75% or 80% homology with the C-terminal seven cysteine domain present in the active forms of human OP-1 (i.e., residues 330-431, as shown in SEQ ID NO: 2 of U.S. Pat. No. 5,266,683). In other preferred embodiments, the renal therapeutic agents useful herein include therapeutically effective proteins in which the amino acid sequences comprise a sequence sharing at least 60% amino acid sequence identity and, preferably, 65% or 70% identity with the C-terminal seven cysteine domain present in the active forms of human OP-1. As will be understood by those skilled in the art, homologous or functionally equivalent sequences include functionally equivalent arrangements of the cysteine residues within the conserved cysteine skeleton, including amino acid insertions or deletions which alter the linear arrangement of these cysteines, but do not materially impair their relationship in the folded structure of the dimeric protein, including their ability to form such intra- or inter-chain disulfide bonds as may be necessary for biological activity. To determine the degree of homology of a candidate amino acid sequence to the C-terminal seven cysteine skeleton of human OP-1, the candidate sequence and the reference sequence are first aligned using an alignment algorithm, such as the dynamic programming algorithm described in Needleman et al., J. Mol. Biol. 48:443 (1970), or the Align Program, a commercial software package produced by DNAstar, Inc., the teachings of which are incorporated by reference herein. After the initial alignment is made, it may then be refined by comparison to the sequences of other members of the OP/BMP family of related proteins. Once the alignment between the candidate and reference sequences is made and refined, a percent homology score is calculated. The individual amino acids of each sequence are compared sequentially according to their similarity to each other. Similarity factors include similar size, shape and electrical charge. One particularly preferred method of determining amino acid similarities is the PAM250 matrix described in Dayhoff et al. (1978), Atlas of Protein Sequence and Structure Vol. 5 (Suppl. 3), pp. 354-352, Natl. Biomed. Res. Found., Washington, D.C., incorporated by reference herein. A similarity score is first calculated as the sum of the aligned pair-wise amino acid similarity scores. Insertions and deletions are ignored for the purposes of percent homology and identity. Accordingly, gap penalties are not used in this calculation. The raw score is then normalized by dividing it by the geometric mean of the scores of the candidate compound and the seven cysteine skeleton of hOP-1. The geometric mean is the square root of the product of these scores. The normalized raw score is the percent homology.

Thus, amino acid sequence "homology" is understood herein to include both amino acid sequence identity and similarity and, as used herein, a percentage "homology" between two amino acid sequences indicates the percentage of amino acid residues which are identical or similar between the sequences. "Similar" residues are "conservative substitutions" which fulfill the criteria defined for an "accepted point mutation" in Dayhoff et al. (1978), supra.. Thus, "conservative substitutions" are residues that are physically or functionally similar to the corresponding reference residues, having similar size, shape, electric charge, and/or chemical properties such as the ability to form covalent or hydrogen bonds, or the like. Examples of conservative substitutions include the substitution of one amino acid for another with similar characteristics, e.g., substitutions within the following groups: (a) valine, glycine; (b) glycine, alanine; (c) valine, isoleucine, leucine; (d) aspartic acid, glutamic acid; (e) asparagine, glutamine; (f) serine, threonine; (g) lysine, arginine, methionine; and (h) phenylalanine, tyrosine. The term "conservative substitution" or "conservative variation" also includes the use of a substituted amino acid in place of an unsubstituted parent amino acid in a given polypeptide chain, provided that the resulting substituted polypeptide chain also has therapeutic efficacy in the present invention.

The renal therapeutic agents of the invention are also characterized by biological activities which may be readily ascertained by those of ordinary skill in the art. Specifically, a renal therapeutic agent of the present invention is (a) capable of inducing chondrogenesis in the Reddi-Sampath ectopic bone assay (Sampath and Reddi (1981), Proc. Natl. Acad. Sci. (USA) 78:7599-7603) or a substantially equivalent assay, (b) capable of significantly preventing, inhibiting, delaying or alleviating the permanent or progressive loss of renal function which may result from acute renal failure in a standard animal model of acute renal failure, or (c) capable of causing a clinically significant improvement in a standard marker of renal function when administered to a mammal in, or at risk of, acute renal failure.

The Reddi-Sampath ectopic bone assay is well known in the art as an assay of chondrogenic activity. The assay, which can be easily performed, is described and discussed in, for example, Sampath and Reddi (1981), Proc. Natl. Acad. Sci. (USA) 78:7599-7603; and Wozney (1989), "Bone Morphogenetic Proteins," Progress in Growth Factor Research 1:267-280. Many equivalent assays, using other animals and tissue sites, may be employed or developed by those of skill in the art to evaluate the biological activity of the renal therapeutic agents of the present invention. See, for example, the bioassays described in U.S. Pat. No. 5,226,683.

The renal therapeutic agents of the present invention also may be tested in animal models of acute renal failure. Mammalian models of acute renal failure in, for example, mice, rats, guinea pigs, cats, dogs, sheep, goats, pigs, cows, horses, and non-human primates, may be created by causing an appropriate direct or indirect injury or insult to the renal tissues of the animal. Animal models of acute renal failure may, for example, be created by inducing in the animal the conditions

or diseases described above as placing a subject "at risk" of acute renal failure. In particularly preferred embodiments, an animal model is employed in which acute renal failure is induced by the controlled administration of nephrotoxic agents (e.g., cisplatin, aminoglycoside antibiotics, heavy metals).

Finally, the renal therapeutic agents of the present invention may be evaluated for their therapeutic efficacy in causing a clinically significant improvement in a standard marker of renal function when administered to a mammalian subject (e.g., a human patient) in, or at risk of, acute renal failure. Such markers of renal function are well known in the medical literature and include, without being limited to, rates of increase in BUN levels, rates of increase in serum creatinine, static measurements of BUN, static measurements of serum creatinine, glomerular filtration rates (GFR), ratios of BUN/creatinine, serum concentrations of sodium (Na+), urine/plasma ratios for creatinine, urine/plasma ratios for urea, urine osmolality, daily urine output, and the like (see, for example, Anderson and Schrier (1994), in Harrison's Principles of Internal Medicine, 13th edition, Isselbacher et al., eds., McGraw Hill Text, New York; Kumar and Stein (1994), in Internal Medicine, 4th Edition, J.H. Stein, ed., Mosby-Year Book, Inc. St. Louis).

The renal therapeutic agents contemplated herein can be expressed from intact or truncated genomic or cDNA or from synthetic DNAs in prokaryotic or eukaryotic host cells. The dimeric proteins can be isolated from the culture media and/or refolded and dimerized in vitro to form biologically active compositions. Heterodimers can be formed in vitro by combining separate, distinct polypeptide chains. Alternatively, heterodimers can be formed in a single cell by co-expressing nucleic acids encoding separate, distinct polypeptide chains. See, for example, WO93/09229, or U.S. Pat. No. 5,411,941, for several exemplary recombinant heterodimer protein production protocols. Currently preferred host cells include, without limitation, prokaryotes including E. coli, or eukaryotes including yeast, Saccharomyces, insect cells, or mammalian cells, such as CHO, COS or BSC cells. One of ordinary skill in the art will appreciate that other host cells can be used to advantage. Detailed descriptions of the proteins useful in the practice of this invention, including how to make, use and test them for chondrogenic activity, are disclosed in numerous publications, including U.S. Pat. Nos. 5,266,683 and 5,011,691, the disclosures of which are herein incorporated by reference.

C. Subjects for Treatment

As a general matter, the methods of the present invention may be utilized for any mammalian subject in, or at risk of, acute renal failure. Mammalian subjects which may be treated according to the methods of the invention include, but are not limited to, human subjects or patients. In addition, however, the invention may be employed in the treatment of domesticated mammals which are maintained as human companions (e.g., dogs, cats, horses), which have significant commercial value (e.g., dairy cows, beef cattle, sporting animals), which have significant scientific value (e.g., captive or free specimens of endangered species), or which

otherwise have value. In addition, as a general matter, the subjects for treatment with the methods of the present invention need not present indications for treatment with the agents of the invention other than those associated with acute renal failure or risk of acute renal failure. That is, the subjects for treatment may be otherwise free of indications for treatment with the agents of the invention. In some number of cases, however, the subjects may present with other symptoms (e.g., osteodystrophy) for which renal therapeutic agent treatment would be indicated. In such cases, the renal therapeutic agent treatment should be adjusted accordingly so to avoid excessive dosing.

One of ordinary skill in the medical or veterinary arts is trained to recognize subjects in, or at risk of, acute renal failure, and a detailed scientific literature has developed around the subject. See, for example, Anderson and Schrier (1994), in Harrison's Principles of Internal Medicine, 13th edition, Isselbacher et al., eds., McGraw Hill Text, New York; Kumar and Stein (1994), in Internal Medicine, 4th Edition, J.H. Stein, ed., Mosby-Year Book, Inc. St. Louis.

Preferably, a diagnosis that a subject is in acute renal failure, or at risk of entering acute renal failure, is made on the basis of serial blood tests measuring, among other factors, the circulating levels of serum creatinine and blood urea nitrogen. Such "serial" blood tests may be taken every few hours immediately upon admittance of an undiagnosed patient presenting with symptoms of acute renal failure. More typically, however, consecutive serial blood tests are separated by a period of at least 6 hours, not more than 72 hours, and preferably 12-24 hours. On the basis of two or more blood tests within a 24 or 72 hour period, it is possible to calculate a rate of increase of serum creatinine or BUN.

Finally, it should be noted that subjects possessing a single kidney, irrespective of the manner of loss of the other kidney (e.g., physical trauma, surgical removal, birth defect), may be considered to be at increased risk of acute renal failure. This is particularly true for those subjects in which one kidney has been lost due to a disease or condition which may afflict the remaining kidney. Similarly, subjects which are already recipients of a renal transplant, or which are receiving chronic dialysis (e.g., chronic hemodialysis or continuous ambulatory peritoneal dialysis) may be considered to be at increased risk of acute renal failure. Therefore, for these subjects, the clinical indications discussed above may need to be more carefully monitored, and earlier or more aggressive intervention with renal therapeutic agent treatment may be advisable.

In another aspect, the OP/BMP renal therapeutic agents of the invention are useful in reducing inflammation and neutrophil-mediated tissue damage. This is demonstrated in Example 3, below, in which it is seen that systemically administered OP-1 reduces inflammation, the accumulation and activity of neutrophils, and neutrophil-mediated damage in a rat ischemia-reperfusion injury model of ARF. Thus, the OP/BMP therapeutic agents are shown to be useful in reducing inflammation, the accumulation and activity of neutrophils, and neutrophil-mediated damage in injured or damaged epithelial tissue, and particularly renal epithelial tissue. In this

same respect, the OP/BMP therapeutic agents are also shown to be useful in reducing the expression of ICAMs, particularly ICAM-1, when administered systemically. Thus, the OP/BMP therapeutics are shown to be useful in reducing ICAM expression in epithelial issue, particularly renal epithelial tissue.

In yet another aspect, the OP/BMP renal therapeutic agents of the invention are useful for inhibiting apoptosis of cells in damaged or injured tissues. This is also demonstrated in Example 3, below, in which it is seen that systemically administered OP-1 reduces the number of apoptotic cells in a rat ischemia-reperfusion injury model of ARF. Thus, the OP/BMP therapeutic agents are shown to be useful in inhibiting apoptosis of damaged or injured epithelial tissues, particularly renal epithelium.

D. Formulations and Methods of Treatment

The renal therapeutic agents of the present invention may be administered by any route which is compatible with the particular renal therapeutic agent employed. Thus, as appropriate, administration may be oral or parenteral, including intravenous, intraperitoneal, and renal intracapsular routes of administration. In addition, administration may be by periodic injections of a bolus of the renal therapeutic agent, or may be made more continuous by intravenous or intraperitoneal administration from a reservoir which is external (e.g., an i.v. bag) or internal (e.g., a bioerodable implant or implanted pump).

The renal therapeutic agents of the invention may be provided to an individual by any suitable means, preferably directly (e.g., locally, as by injection or topical administration to a tissue locus) or systemically (e.g., parenterally or orally). Where the agent is to be provided parenterally, such as by intravenous, subcutaneous, or intramuscular, administration, the agent preferably comprises part of an aqueous solution. The solution is physiologically acceptable so that in addition to delivery of the desired agent to the subject, the solution does not otherwise adversely affect the subject's electrolyte and/or volume balance. The aqueous medium for the agent thus may comprise normal physiologic saline (e.g., 9.85% NaCl, 0.15M, pH 7-7.4).

If desired, a given renal therapeutic agent or other agent may be made more soluble by association with a suitable molecule. For example, association of a mature OP/BMP dimer with an OP/BMP pro domain results in the pro form of the renal therapeutic agent which typically is more soluble or dispersible in physiological solutions than the corresponding mature form. In fact, endogenous members of the OP/BMP family are thought to be transported (e.g., secreted and circulated) in the mammalian body in this form. This soluble form of the protein can be obtained from culture medium of OP/BMP-secreting mammalian cells, e.g., cells transfected with nucleic acid encoding and competent to express the protein. Alternatively, a soluble species can be formulated by complexing the mature dimer (or an active fragment thereof) with a pro domain or a solubility-enhancing fragment thereof (described more fully below). Another molecule capable of enhancing solubility and particularly useful for oral administrations, is casein. For

example, addition of 0.2% casein increases solubility of the mature active form of OP-1 by 80%. Other components found in milk and/or various serum proteins also may be useful.

Useful solutions for parenteral administration may be prepared by any of the methods well known in the pharmaceutical art, described, for example, in Remington's Pharmaceutical Sciences (Gennaro, A., ed.), Mack Pub., 1990.

Alternatively, the agents described herein may be administered orally. Oral administration of proteins as therapeutics generally is not practiced as most proteins are readily degraded by digestive enzymes and acids in the mammalian digestive system before they can be absorbed into the bloodstream. However, the renal therapeutic agents described herein typically are acid stable and protease-resistant (see, for example, U.S. Pat. No. 4,968,590). In addition, at least one of these renal therapeutic agents, OP-1, has been identified in mammary gland extract, colostrum and 57-day milk. Moreover, the OP-1 purified from mammary gland extract is therapeutically efficacious and also is detected in the bloodstream. Finally, soluble form OP-1, e.g., mature OP-1 associated with the pro domain, is therapeutically efficacious. These findings, as well as those disclosed in the examples below, indicate that oral and parenteral administration are viable means for administering the renal therapeutic agents of the invention to an individual. In addition, while the mature forms of certain renal therapeutic agents described herein typically are sparingly soluble, the form found in milk (and mammary gland extract and colostrum) is readily soluble, probably by association of the mature, therapeutically efficacious form with part or all of the pro domain of the intact sequence and/or by association with one or more milk components. Accordingly, the compounds provided herein also may be associated with molecules capable of enhancing their solubility in vitro or in vivo.

The compounds provided herein also may be associated with molecules capable of targeting the renal therapeutic agent to the desired tissue. For example, an antibody, antibody fragment, or other binding protein that interacts specifically with a surface molecule on cells of the desired tissue, may be used. Useful targeting molecules may be designed, for example, using the single chain binding site technology disclosed, for example, in U.S. Pat. No. 5,091,513.

As will be appreciated by one of ordinary skill in the art, the formulated compositions contain therapeutically effective amounts of the renal therapeutic agent. That is, they contain amounts which provide appropriate concentrations of the agent to the renal tissues for a time sufficient to prevent, inhibit, delay or alleviate permanent or progressive loss of renal function, or otherwise provide therapeutic efficacy. As will be appreciated by those skilled in the art, the concentration of the compounds described in a therapeutic composition of the present invention will vary depending upon a number of factors, including the biological efficacy of the selected agent, the chemical characteristics (e.g., hydrophobicity) of the compounds employed, the formulation of the compound excipients, the administration route, and the treatment envisioned, including whether the active ingredient will be administered directly into a kidney or renal capsule,

or whether it will be administered systemically. The preferred dosage to be administered also is likely to depend on such variables such as the condition of the renal tissues, extent of renal function loss, and the overall health status of the particular subject. Dosages are preferably administered continuously, but daily, multi-weekly, weekly or monthly dosages may also be employed. For subjects which would otherwise require continuous, bi-weekly or tri-weekly hemodialysis sessions, continuous, bi-weekly or tri-weekly intravenous or intraperitoneal infusions are not considered unduly inconvenient. In addition, in order to facilitate frequent infusions, implantation of a semi-permanent stent (e.g., intravenous, intraperitoneal or intracapsular) may be advisable.

The renal therapeutic agents of the invention may, of course, be administered alone or in combination with other molecules known to be beneficial in the treatment of the conditions described herein. When used in combination with other agents, it may be advisable to alter the dosages of the renal therapeutic agents of the present invention accordingly.

Thus, in another aspect, the present invention provides for the use of the OP/BMP renal therapeutic agents of the invention in the manufacture of medicaments for the treatment of any of the above-described conditions.

Practice of the invention, including additional preferred aspects and embodiments thereof, will be still more fully understood from the following examples, which are presented herein for illustration only and should not be construed as limiting the invention in any way.

Example 1

To examine the ability of OP-1 to beneficially affect the course of renal function after an acute ischemic insult to the kidney, the effect of OP-1 on creatinine levels was examined in rats in which ischemic renal damage was induced by temporarily preventing renal blood flow. In this model system, both renal arteries are clamped for 60 minutes. OP-1 is administered into the tail vein of each rat at 0.25 μg/kg at the following four times: 10 minutes before artery clamping, and then again at each of 24, 48, and 72 hours after the ischemic injury. As shown in Figure 1, the creatinine level of placebo-treated rats rises rapidly after injury, peaks at the 24 hour measurement, and is back to normal by the 18th day post-injury. In contrast, OP-1 treatment is associated with a creatinine level which peaks at the 24 hour measurement to a level only half that of the placebo group, after which it begins to decrease. For each of the four post-injury days on which measurements were made, the creatinine level of the placebo treated rats was at least twice as high as that of the OP-1 treated animals. These results indicate that OP-1 may limit the damage caused to renal tissue by an ischemic insult, and may also speed the functional recovery of the kidney.

Example 2

To study the effect of post-injury OP-1 treatment on renal function in another animal model of renal failure, rats with a single kidney were treated with OP-1 2 days after norepinephrine-induced renal damage. In the model system (further described in Conger et al., Kidney Intl. 40:21-28, 1991), each rat has a kidney removed 7 days prior to renal injury, which is induced by intra-arterial infusion of norepinephrine for 90 minutes. Two days after norepinephrine administration, OP-1 is administered intravenously at 0.25 mg/kg.

The upper table of Figure 2 presents creatinine levels of vehicle- and OP-1 treated rats. On the fourth day after injury, the animals which had been treated with OP-1 on the second day after injury had creatinine levels which are significantly lower than the levels of the vehicle-treated controls. The data presented in the lower table of Figure 2 shows that OP-1 treatment was also associated with improvements in renal blood flow, GFR, and urine flow as compared to controls.

Example 3

Further experiments, using ischemia-reperfusion injury as a model of acute renal failure in rats, were conducted as follows:

Animal Surgical Procedures and Experimental protocols.

Wistar male rats of 200-250 g (Pliva Breeding Laboratory, Zagreb, Croatia) were fasted for 12 h prior to surgery. After intraperitoneal administration of ketamin (20 mg/kg) anesthetic, both renal arteries were dorsally occluded for 60 minutes with microaneurysm clamps (Roboz). Vehicle buffer or OP-1 containing 20 mM sodium acetate buffer (500µl) were administered via the tail vein. All animals were subjected to intraperitoneal administration of 1-3 ml of pre-warmed (37°C) saline (0.9% NaCl) to compensate for any fluid loss during the surgery. Experiments were blind, and rats were terminated at different time intervals ranging from 30 min to 18 days. Blood samples (0.5 ml) were obtained from the orbital plexus at 0 h, 24 h, 48 h, and 72 h and, in some cases, at 30 min, 2 h, 8 h, and 96 h, and 18 days following reperfusion. For GFR measurements, urine was collected from metabolic cages for 24 h as previously described (Vukicevic et al. (1989) Bone Miner. 6:125-39). Serum and urine creatinine was measured by the Jaffe method (Whelton et al. (1994) in Clinical Chemistry, Burtis and Ashwood, eds., Amer. Assn. Clin. Chem.), blood urea nitrogen (BUN) was measured by glutamate dehydrogenase UV procedure, phosphorus was measured by a molybdate method, and calcium was measured by an o-cresolphtaleine method. Serum electrolytes were measured by indirect potentiometry. Fifteen independent experiments with a total of 456 rats were performed. In a prophylactic model, OP-1 was administered 10 minutes prior to surgery and then at 24 h, 48 h, and 72 h thereafter. In a therapeutic model, the first OP-1 injection was administered either at 1 h or 16 h after reperfusion, followed by injections at 24 h intervals up to 96 h following reperfusion.

Histomorphometry and immunocytochemistry

At sacrifice, each kidney was split longitudinally and one half was fixed in 4% paraformaldehyde. Two consecutive sections from paraffin embedded blocks were stained with H&E and PAS. The morphometric measurements were done on longitudinal sections using an eye-piece grid that contains 100-point grid lines under a Nikon microscope (Nikon Optiphot-2; MVI, Avon, MA). The grid was calibrated by a stage micrometer and the area of a specific feature was measured by counting the number of intersects (points or hits) that were superimposed on the tissue of interest. All counts were done through a 1x objective lens. Each point or hit represents an area of 0.61 mm^2. Verification of features were done at a higher magnification, if they could not be discerned through the 1x objective lens.

Morphometric variables included total tissue area, dilated tubular area, plugged tubular area, infarcted area, and necrotic area. A dilated tubule was defined as a tubular structure having distended lumen and identifiable epithelial cells. A plugged tubule was defined as a tubular structure having debris-filled lumen and identifiable epithelial cells. An infarcted area was defined as a portion or a zone of the kidney showing identifiable tissue profiles and lost cellular details, which was stained bright pink by H&E stain, and which was associated with regional congestion in the outer medulla. A necrotic area was defined as a portion or a zone of the kidney located in the infarcted area showing loss of both tissue profits and cellular details.

Immunocytochemistry was performed using the immunoperoxidase detection system (Zymed, San Francisco, CA). The following monoclonal antibodies were used: PCNA (proliferating cell nuclear antigen; Dako, Denmark), smooth muscle α-actin (SMA) (Dako, Denmark), smooth muscle myosin IgG (BTI, Stoughton, MA) and ICAM (CD 54; Dako, Denmark). A minimum of 3000 cells were counted per kidney section stained for PCNA and SMA, and the number of positive cells was expressed as a percentage of total counted cells in the subdivisions of cortex, and/or S_3 zone.

Apoptotic cells were detected by a TACS 2 TdT *in situ* apoptosis system (Trevigen, Gaithersburg, MD). The total number of apoptotic cells were counted in cortex and medulla in three sections per kidney from eight independent animals per group, sacrificed at days 1, 2, 3, and 5 following reperfusion.

Neutrophil accumulation and activity

The neutrophil infiltration was determined using naphthol AS-D chloroacetate esterase staining (Sigma) on histologic sections. The neutrophils were counted in the cortex and S_3 zone (outer medulla and inner cortex) using an ocular grid. Data were expressed as number of neutrophils per mm^2, evaluated on 100 high power fields per section, two sections per animal from 8 independent rats at 24 h following injury. In addition, neutrophil activity was determined at 24 h following injury by myeloperoxidase activity (MPO). For MPO, kidneys were extracted in 0.5% HTAB (Sigma) in 50 mM KPO_4 buffer, pH 6.0, homogenized for 10 min, sonicated for 5

min and, finally, the lysate was centrifuged for 60 min at 20,000xg. Ten µl of extract was incubated with 1 ml of 50 mM KPO_4 buffer containing 0.167 mg/ml O-dianisidine (Sigma) and 0.0005% H_2O_2 at 25°C. Absorbance was determined at 460 nm using a myeloperoxidase standard (Oxis) and normalized to kidney wet weight.

Statistical analysis

Two way analysis of variance and post hoc analysis with Duncan's multiple range test were performed to determine the effects of treatment and time on biochemical and histological parameters. Mann-Whitney-U tests & Chi-tests were used to determine the significance of differences in results between selected groups.

Prophylactic and Therapeutic Effects

In a typical experiment, at 24 h post reperfusion, serum creatinine (Cr) and blood urea nitrogen (BUN) levels were increased 8 to 10 times above normal values in rats with acute renal failure that received vehicle acetate buffer (500 µl; pH 4.5). Intravenous injection of 250 µg OP-1/kg at 10 minutes prior to clamping and then at 24 h intervals up to 72 h post reperfusion, dramatically decreased the mortality rate and strongly suppressed the elevation of serum Cr, BUN, phosphorus and potassium values, as compared to vehicle-treated rats. Serum sodium and chloride values were unchanged in all animals. Vehicle alone did not influence the kidney function in rats with ARF as compared to ARF rats that received none. The OP-1 dose which successfully protected the kidney against ischemic injury varied with age of the animal and severity of ischemia and reperfusion, and was within a range of 50 - 250 µg/kg. GFR measured at 24 h after reperfusion was significantly higher in OP-1 treated rats than in vehicle-treated rats. Specifically, sham rats had a GFR (ml/min) of 4.7±0.8; vehicle-treated ARF rats had a GFR of 0.25±0.11; and OP-1 treated rats had a GFR of 0.45±0.17 ($p<0.05$ vs ARF). To examine the therapeutic potential of OP-1 for the treatment of established ARF, OP-1 was administered either at 1 h or 16 h following reperfusion. Both OP-1 treatment groups showed lower serum Cr and BUN values. The reduction of serum Cr and BUN was more dramatic in rats with OP-1 administered at 1 h than those receiving OP-1 at 16 h post reperfusion. These results indicate that OP-1 protects against loss of kidney function in ischemia as measured by serum biochemical parameters.

Protection against kidney damage

Histological analysis of kidney sections from rats with ARF indicated that morphologic changes had occurred in the S_3 zone. At 2 h after reperfusion, vehicle treated kidneys showed signs of congestion which led to large areas of parenchymal infarction and necrosis when examined at 24 h, 72 h, and 120 h after reperfusion. Animals given OP-1 prior to ischemia, however, exhibited little or no congestion and had less infarction; 28 of 32 vehicle-treated kidneys and 16 of 32 OP-1 treated kidneys had white infarction (necrosis), and 26 of 32 vehicle-treated and 14 of 32 OP-1 treated rats had red infarction. Histomorphometric analyses showed that 6 and

11% of the kidney section area in vehicle-treated rats and 0 and 0.3% of OP-1 treated rats had white or red infarction, respectively. In the kidney area affected by infarction, approximately 50% of tubules were dilated both in vehicle and OP-1 treated rats at 1 to 5 days following injury. However, fewer tubules plugged with desquamated epithelial cells, cell debris, and cast matrix were found in OP-1 treated rats as compared with vehicle-treated animals on days 2 and 5 post injury. The analysis of peritubular capillary-derived smooth muscle cells revealed that at 24 h post-injury, approximately 5-fold more cells in the S_3 zone of OP-1 treated kidneys expressed α-actin as compared to vehicle treated kidneys, suggesting that OP-1 therapy supports the maintenance of a vascular smooth muscle phenotype. Cell proliferation evaluated by PCNA staining indicated an increase in proximal tubule cell growth in the cortex and outer medulla of OP-1 treated rats. In animals treated 1 h after reperfusion, OP-1 had a similar protective action on kidney histology. In animals treated 16 h after reperfusion, the structural damage was similar to vehicle-treated animals at 24 h following injury.

Suppression of Inflammation

As ICAM-1 has been reported to play an important role during the onset of ARF, we determined the effect of OP-1 on the expression of ICAM-1 at different time points following ischemia and reperfusion. OP-1 treatment 10 min prior to ischemia attenuated the expression of ICAM-1 as determined by molecular and histochemical analyses of kidneys obtained at 30 min, 2 h and 8 h following reperfusion. Significant neutrophil accumulation in vehicle-treated rats was observed at 24 h following reperfusion in the S_3 zone. In contrast, rats treated with OP-1 had dramatically decreased neutrophil accumulation (232 ± 47 cells/mm^2 in vehicle vs 9 ± 3 cells/mm^2 in OP-1 group; $n = 8$, $p < 0.01$). Neutrophil activity in the kidney was monitored by measuring the total tissue myeloperoxidase activity (MPO). Administration of OP-1 1 h following reperfusion decreased the MPO activity approximately 3-fold as determined at 24 h post reperfusion (MPO/μg/wet kidney: 37.3 ± 16.5 in vehicle vs 12.8 ± 7.6 in OP-1 treated kidneys; $n = 8$; $p < 0.01$).

Reduction of Apoptosis

At days 1 and 2 following injury, there were no differences in the number of apoptotic cells in the medulla of both OP-1 and vehicle-treated kidneys. However, a reduction in the number of apoptotic cells was observed in the cortex of OP-1 treated rats. Five days after injury, OP-1 treatment resulted in a dramatic reduction of apoptotic cells attached to the basement membrane of tubules of both the cortex and medulla, whereas numerous apoptotic cells were observed in the lumen of the renal tubules of vehicle-treated rats.

Equivalents

The invention may be embodied in other specific forms without departing from the spirit or essential characteristics thereof. The foregoing embodiments are therefore to be considered in

all respects illustrative rather than limiting on the invention described herein. Scope of the invention is thus indicated by the appended claims rather than by the foregoing description, and all changes which come within the meaning and range of equivalency of the claims are intended to be embraced therein.

CLAIMS

What is claimed is:

1. A method of treatment for a mammal in, or at risk of, acute renal failure comprising

administering to said mammal a therapeutically effective amount of an OP/BMP renal therapeutic agent.

2. A method of treatment to delay the need for, or reduce the frequency of, dialysis treatments of a mammal, the method comprising

administering to said mammal a therapeutically effective amount of an OP/BMP renal therapeutic agent.

3. A method of reducing inflammation, the accumulation of neutrophils, and/or neutrophil-mediated damage in a mammalian tissue which has been damaged or injured, or which is at risk of damage or injury, comprising

administering to said mammal a therapeutically effective amount of an OP/BMP renal therapeutic agent.

4. A method of inhibiting apoptosis of cells in a mammalian tissue which has been damaged or injured, or which is at risk of damage or injury, comprising

administering to said mammal a therapeutically effective amount of an OP/BMP renal therapeutic agent.

5. A method as in any one of claims 1-4 wherein said renal therapeutic agent comprises a polypeptide consisting of at least a C-terminal cysteine domain of a protein selected from the group consisting of a pro form, a mature form, and a soluble form of a polypeptide selected from the group consisting of OP-1, OP-2, OP-3, BMP2, BMP3, BMP4, BMP5, BMP6, and BMP9.

6. A method as in claim 5 wherein said renal therapeutic agent comprises a polypeptide consisting of at least a C-terminal cysteine domain of a protein selected from the group consisting of a pro form, a mature form, and a soluble form of human OP-1.

7. A method as in any one of claims 1-4 wherein said renal therapeutic agent comprises a polypeptide having at least 70% homology with an amino acid sequence of a seven-cysteine domain of human OP-1.

8. A method as in claim 7 wherein said polypeptide has at least 75% homology with an amino acid sequence of a seven-cysteine domain of human OP-1.

9. A method as in claim 7 wherein said polypeptide has at least 80% homology with an amino acid sequence of a seven-cysteine domain of human OP-1.

10. A method as in claim 7 wherein said polypeptide has at least 60% identity with an amino acid sequence of a seven-cysteine domain of human OP-1.

11. A method as in claim 7 wherein said polypeptide has at least 65% identity with an amino acid sequence of a seven-cysteine domain of human OP-1.

12. A method as in claim 7 wherein said polypeptide has at least 70% identity with an amino acid sequence of a seven-cysteine domain of human OP-1.

13. A method as in any one of claims 5-12 wherein said renal therapeutic agent

(a) induces chondrogenesis in an ectopic bone assay;

(b) prevents, inhibits, delays or alleviates loss of renal function resulting from acute renal failure in an animal model of acute renal failure; or

(c) causes a clinically significant improvement in a standard marker of renal function when administered to a mammal in, or at risk of, acute renal failure.

14. A method as in any one of claims 1-4 wherein said renal therapeutic agent is selected from the group consisting of human osteogenic proteins and human bone morphogenic proteins.

15. A method as in any one of claims 1-2 wherein

serial determination of BUN in said mammal indicates a rate of increase in BUN of at least 2 to 4 mmol/L/day (5 to 10 mg/dL/day).

16. A method as in any one of claims 1-2 wherein

serial determination of BUN in said mammal indicates a rate of increase in BUN of at least 4 to 8 mmol/L/day (10 to 20 mg/dL/day).

17. A method as in any one of claims 1-2 wherein

serial determination of serum creatinine in said mammal indicates a rate of increase in serum creatinine of at least 20 to 40 μmol/L/day (0.25 to 0.5 mg/dL/day).

18. A method as in any one of claims 1-2 wherein

serial determination of serum creatinine in said mammal indicates a rate of increase in serum creatinine of at least 40 to 80 μmol/L/day (0.5 to 1.0 mg/dL/day).

19. A method as in any one of claims 1-2 wherein

said mammal is afflicted with a condition selected from the group consisting of pre-renal causes of acute renal failure, post-renal causes of acute renal failure, and intrinsic renal causes of acute renal failure.

20. A method as in claim 19 wherein

said mammal is afflicted with a pre-renal cause of acute renal failure selected from the group consisting of decreased cardiac output, hypovolemia, volume redistribution, and altered vascular resistance.

21. A method as in claim 19 wherein

said mammal is afflicted with a post-renal cause of acute renal failure selected from the group consisting of ureteral, pelvic and bladder obstructions.

22. A method as in claim 19 wherein

said mammal is afflicted with an intrinsic renal cause of acute renal failure selected from the group consisting of abnormalities of the vasculature, abnormalities of the glomeruli, acute interstitial nephritis, intratubular obstruction, and acute tubular necrosis.

23. A method as in any one of claims 1-2 wherein

said mammal is a kidney transplant recipient.

24. A method as in any one of claims 1-2 wherein

said mammal possesses only one kidney.

25. A method as in any one of claims 1-4 wherein said administration is oral.

26. A method as in any one of claims 1-4 wherein said administration is parenteral.

27. A method as in any one of claims 1-4 wherein said administration is intravenous.

28. A method as in any one of claims 1-4 wherein said administration is intraperitoneal.

29. A method as in any one of claims 1-4 wherein said administration is into the renal capsule.

30. A method as in claim 26 wherein a stent has been implanted into said mammal for said administration.

31. A method as in claim 30 wherein said stent is an intravenous stent.

32. A method as in claim 30 wherein said stent is an intraperitoneal stent.

33. A method as in claim 30 wherein said stent is a renal intracapsular stent.

34. A method as in claim 26 wherein said administration is by an implanted device.

35. A method as in any one of claims 1-4 wherein said administration is daily for a period of at least about one week.

36. A method as in any one of claims 1-4 wherein said administration is at least once a week for a period of at least about one month.

37. A method as in any one of claims 1-4 wherein said renal therapeutic agent is administered at a dosage of about 0.01-1000 μg/kg body weight of said mammal.

38. A method as in claim 37 wherein said renal therapeutic agent is administered at a dosage of about 0.1-100 μg/kg body weight of said mammal.

39. Use of an OP/BMP renal therapeutic agent in the manufacture of a medicament for the treatment for a mammal in, or at risk of, acute renal failure.

40. Use of an OP/BMP renal therapeutic agent in the manufacture of a medicament to delay the need for, or reduce the frequency of, dialysis treatments of a mammal.

41. Use of an OP/BMP renal therapeutic agent in the manufacture of a medicament for reducing inflammation, the accumulation of neutrophils, and/or neutrophil-mediated damage in a mammalian tissue which has been damaged or injured, or which is at risk of damage or injury.

42. Use of an OP/BMP renal therapeutic agent in the manufacture of a medicament for inhibiting apoptosis of cells in a mammalian tissue which has been damaged or injured, or which is at risk of damage or injury.

43. A use as in any one of claims 39-42 wherein said renal therapeutic agent comprises a polypeptide consisting of at least a C-terminal cysteine domain of a protein selected from the group consisting of a pro form, a mature form, and a soluble form of a polypeptide selected from the group consisting of OP-1, OP-2, OP-3, BMP2, BMP3, BMP4, BMP5, BMP6, and BMP9.

44. A use as in claim 43 wherein said renal therapeutic agent comprises a polypeptide consisting of at least a C-terminal cysteine domain of a protein selected from the group consisting of a pro form, a mature form, and a soluble form of human OP-1.

45. A use as in any one of claims 39-42 wherein said renal therapeutic agent comprises a polypeptide having at least 70% homology with an amino acid sequence of a seven-cysteine domain of human OP-1.

46. A use as in claim 45 wherein said polypeptide has at least 75% homology with an amino acid sequence of a seven-cysteine domain of human OP-1.

47. A use as in claim 45 wherein said polypeptide has at least 80% homology with an amino acid sequence of a seven-cysteine domain of human OP-1.

48. A use as in claim 45 wherein said polypeptide has at least 60% identity with an amino acid sequence of a seven-cysteine domain of human OP-1.

49. A use as in claim 45 wherein said polypeptide has at least 65% identity with an amino acid sequence of a seven-cysteine domain of human OP-1.

50. A use as in claim 45 wherein said polypeptide has at least 70% identity with an amino acid sequence of a seven-cysteine domain of human OP-1.

51. A use as in any one of claims 43-50 wherein said renal therapeutic agent

(a) induces chondrogenesis in an ectopic bone assay;

(b) prevents, inhibits, delays or alleviates loss of renal function resulting from acute renal failure in an animal model of acute renal failure; or

(c) causes a clinically significant improvement in a standard marker of renal function when administered to a mammal in, or at risk of, acute renal failure.

52. A use as in any one of claims 39-42 wherein said renal therapeutic agent is selected from the group consisting of human osteogenic proteins and human bone morphogenic proteins.

FIG. 1

Creatinine Levels (mg/dL)

	Vehicle (N=8)	OP-1 (N=7)
Day 0	0.55	0.57
Day 2	3.69	3.56
Day 4	2.78	1.59

Hemodynamic parameters (day 4)

	Vehicle (N=8)	OP-1(N=7)
Renal blood flow	5.23	7.97
GFR (ml/min)	0.453	0.731
Urine flow (ul/min)	10.6	16.0
Blood pressure (mm Hg)	119.5	119.9
Heartrate (bpm)	345	338.6

Figure 2

COMBINED DECLARATION AND POWER OF ATTORNEY FOR PATENT APPLICATION

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am an original, first and sole inventor of the subject matter (an original, first and joint inventor) which is claimed and for which a utility patent is sought on the invention entitled:

THERAPIES FOR ACUTE RENAL FAILURE

the specification of which:

☐ was filed on _____, as United States non-provisional application U.S.S.N. _____, bearing Attorney Docket No. _____.

☒ is attached hereto.

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, §1.56.

☐ I hereby claim foreign priority benefits under Title 35, United States Code, §119(a)-(d) or §365(b) of any foreign application(s) for patent or inventor's certificate, or §365(a) of any PCT International application designating at least one country other than the United States listed below and have also identified below any foreign application for patent or inventor's certificate or PCT International application having a filing date before that of the application on which priority is claimed.

Appln. Number	Country *(if PCT, so indicate)*	Filing Date *(dd/mm/yy)*	Priority Claimed	
			Yes	No
			☐	☐
			☐	☐
			☐	☐
			☐	☐
			☐	☐
			☐	☐

☒ I hereby claim the benefit under Title 35, United States Code, § 119(e) or §120 of any United States application(s), or §365(c) of any PCT International application(s) designating the United States of America listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, §1.56 which became available between the filing date of the prior application and the national or PCT International filing date of this application:

Application No. *(U.S.S.N.)*	**Filing Date** *(dd/mm/yy)*	**Status** *(Patented, Pending, Abandoned)*
60/045,619	05/05/97	Abandoned

PCT International Applications designating the United States:

PCT Appln No.	US Serial No.	PCT Filing Date
WO 98/50060	PCT/US98/03197	05/05/98

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or patent issued thereon.

Kuber T. Sampath — 12/1/99

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